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WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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Memorandum

SUBJECT: **Ziram** (PC Code: 034805) **Updated Toxicology Disciplinary Chapter for the Reregistration Eligibility Decision Document**

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Action Requested: Review toxicology studies submitted by the registrant and prepare the toxicology chapter to support reregistration Eligibility decision for ziram.

Attached is the updated toxicology chapter summarizing the findings of the toxicology studies.

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1.0 HAZARD CHARACTERIZATION

Ziram, a dimethyl dithiocarbamate fungicide is used to control fungal diseases in various plants including orchard fruits and ornamentals. It also used as an animal repellent and a bactericide/fungicide in paints, paper mills and in cooling towers. The database for ziram is largely complete and provides sufficient information to characterize toxicity. The only data gaps include dominant lethal assay and a metabolite identification study.

In acute studies, ziram exhibits moderate to low toxicity (Toxicity categories II or III) with as oral LD50=320 mg/kg, a dermal LD50>2000 mg/kg and an inhalation LC50 =0.07mg/L. Ziram is severely irritating to ocular tissue (Toxicity Category I) but not irritating to the skin (Toxicity category IV) and is a moderate dermal sensitizer.

The mechanism of ziram-induced toxicity has not been fully investigated. The primary target organs of ziram appear to be the liver and the nervous system, and thyroid. Liver histopathology, sometimes accompanied by increases in hepatic serum enzyme levels, was seen at various doses in the subchronic and chronic rat studies and the carcinogenicity mouse study. Changes in hepatic serum enzyme levels alone were noted in the 21-day rabbit dermal study. Evidence of neurological impairment include: neurological signs including ataxia, salivation, lacrimation, impaired gait, abnormal posture, and decreased absolute brain weights in the acute neurotoxicity rat study; inhibition of brain cholinesterase and brain neurotoxic esterase in the subchronic neurotoxicity rat study; convulsions in a test animal of the high dose treatment group in the chronic toxicity dog study; and decreased brain weights in the carcinogenicity mouse study. Thyroid C-cell hyperplasia in males and prominent thyroid ultimobranchial cysts in both sexes were noted in the chronic rat study. Thyroid C-cell hyperplasia was also observed in male rats in the NTP carcinogenicity study.

Ziram is not significantly absorbed by the skin. Dermal toxicity study supports low dermal absorption: only minimal hepatotoxicity was noted at the limit dose of 1000 mg/kg/day.

Long-term dietary administration of ziram resulted in an increased incidence of benign hemangiomas in male rats only. The levels of the doses tested appeared adequate. No tumors were noted in male or female mice after long-term dietary administration of ziram. In the NTP study, long-term dietary administration of ziram resulted in an increased incidence of thyroid C-cell tumors in male rats and pulmonary alveolar/bronchiolar tumors in female mice. No effect was observed in female rats or male mice. The Cancer Assessment Review Committee classified ziram as "likely to be carcinogenic in humans."

Ziram does show positive evidence of mutagenicity in the Ames test and conflicting evidence of mutagenicity in CHO gene mutation tests and is negative in unscheduled DNA synthesis assays.

The oral rat developmental study did not show an increased susceptibility of the fetus to ziram *in utero*. Diaphragmatic thinning was seen at a dose of 16 mg/kg/day in fetuses while maternal toxicity resulted in reduced food consumption, salivation, and increased water intake during the treatment interval at the same dose.

The oral rabbit developmental study did not reveal an increased susceptibility of the fetus to ziram *in utero*. Increased incidence of resorptions and postimplantation loss was noted at 15 mg/kg/day in fetuses while maternal toxicity resulted in decreased body weight gain at 7.5 mg/kg/day.

A two-generation rat reproduction study did not show an increased susceptibility of offspring. Reduced pup body weights at birth in F_2 pups and during lactation in both F_1 and F_2 pup were noted at a dose of 42.8 mg/kg/day while systemic parental toxicity resulted in reduced body weights, body weight gains, and decreased food consumption in F_0 and F_1 males and females at 37.5 mg/kg/day.

Both the acute and subchronic neurotoxicity studies show that ziram causes adverse effects on the nervous system as noted above. The developmental neurotoxicity study showed an increased potential for quantitative susceptibility of the fetus to ziram at 5 mg/kg/day based on increased motor activity of pups during lactation period. However, the study was deficient in morphometric analysis of brain tissue and statistical analyses of neurobehavioral data. The maternal effects at 32 mg/kg/day included reduced body weight gains and food consumption.

One rat metabolism study showed that ziram was excreted in the expired air, feces, and urine. Excretion of ziram was greatest in the expired air (37-50%) and was associated with both CO_2 and volatile fractions. Urinary excretion: 17-35%. Fecal excretion: 9-18%. Residual radioactivity was low in tissues (<1%) and carcasses (≤1%) in all dose groups. Excretion via air was rapid (24 - 48 hours) and via urine and feces was complete within 72 hours for low-dose groups, but bi-phasic in the high-dose group with excretion peaks at 0-8 hours, 24-72 hours, and 96 hours. No metabolites were identified.

2.0 REQUIREMENTS

The requirements (CFR §158.340, revised as of July 1, 1999) for Food and Non-Food Use for ziram are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Table 1. Toxicology Data Requirements

Test		Technical	
		Required	Satisfied
870.1100	Acute Oral Toxicity	yes	yes
870.1200	Acute Dermal Toxicity	yes	yes
870.1300	Acute Inhalation Toxicity	yes	yes
870.2400	Primary Eye Irritation	yes	yes
870.2500	Primary Dermal Irritation	yes	yes
870.2600	Dermal Sensitization	yes	yes
870.3100	Oral Subchronic (Rodent)	yes	yes
870.3150	Oral Subchronic (Non-Rodent)	yes	yes [a]
870.3200	21-Day Dermal	yes	yes
870.3250	90-Day Dermal	yes	no [b]
870.3465	90-Day Inhalation	no	-
870.3700a	Developmental Toxicity (Rodent)	yes	yes
870.3700b	Developmental Toxicity(Non-rodent)	yes	yes
870.3800	Reproduction	yes	yes
870.4100a	Chronic Toxicity (Rodent)	yes	yes [c]
870.4100b	Chronic Toxicity (Non-rodent)	yes	yes
870.4200a	Oncogenicity (Rat)	yes	yes [c]
870.4200b	Oncogenicity (Mouse)	yes	yes
870.4300	Chronic/Oncogenicity	yes	yes [c]
870.5100/5265	Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300	Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5375	Mutagenicity—Structural Chromosomal Aberrations	yes yes	yes yes
870.5395	Mutagenicity—*in vivo* mammalian cytogenetics	yes yes	no [d] yes
870.5550	Mutagenicity—Other Genotoxic Effects		
870.6100a	Acute Delayed Neurotox. (Hen)	no	-
870.6100b	90-Day Neurotoxicity (Hen)	no	-
870.6200a	Acute Neurotox. Screening Battery (Rat)	yes	yes
870.6200b	90 Day Neuro. Screening Battery (Rat)	yes	yes
870.6300	Developmental Neurotoxicity	yes	yes
870.7485	General Metabolism	yes	no [e]
870.7600	Dermal Penetration	no	yes

a The dog chronic oral toxicity study (870.4100) satisfies the data requirement for 870.3150.

b A data gap will be considered if there is intentional exposure.

c The combined chronic toxicity/oncogenicity study in the rat satisfied the requirements for guidelines 870.4100a, 870.4200a, and 870.4300.

d A dominant lethal study was requested by the Cancer Assessment Review Committee (CARC); Mouse Micronucleus assay was unacceptable/upgradable.

e A metabolite identification study is requested by the CARC.

3.0 DATA GAP(S)

Data gaps include a metabolite identification study, 28-day inhalation study and a dominant lethal study requested by the Cancer Review Assessment Committee (CARC) in order to evaluate any heritable effects via the germ cells as a result of exposure to ziram. In addition, a data gap for a 90-day dermal study will be considered if there is intentional exposure.

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of data base for acute toxicity: The data base for acute toxicity is considered complete and no additional studies are required at this time. Ziram has moderate acute toxicity. It is a toxicity category II/III for oral and dermal exposure, a category II for inhalation exposure, a category I for eye irritation, and a category IV for skin irritation. Ziram is a moderate sensitizer. The acute toxicity data for ziram are summarized below in Table 2.

4.2 Subchronic Toxicity

Adequacy of data base for subchronic toxicity: The data base for subchronic toxicity is considered complete and no additional studies are required at this time. In the rat feeding study, decreased body weights, body weight gains, and food consumption were observed. In addition, microscopic pathological findings of localized areas of epithelial hyperplasia in the stomach and centrilobular necrosis in one liver lobe were noted. The chronic dog study (870.4100b) satisfies the requirement for an oral subchronic non-rodent study (870.3150); signs of toxicity included a convulsive episode in one animal, decreased body weight gains in the females, and histological findings in the male livers. In the rat 21-day dermal study, decreased body weight and food consumption were observed; additionally, changes in clinical pathology parameters [increased GPT (ALT), GOT (AST), bilirubin and cholesterol] indicated minimal hepatotoxicity.

870.3100 90-Day Oral Toxicity - Rat

In a subchronic 13-week feeding study, MRID No. 42450301, male and female Crl:CD(SD)BR rats (10/sex/dose) were administered Ziram (Technical) in the diet at 0, 100, 300, and 1000 ppm. These doses were equivalent to 0, 7.4, 21.4, 67.8 mg/kg/day for males and 0, 8.8, 24.2, and 76.9 mg/kg/day for females.

Ziram treatment for 13 weeks did not produce clinical signs of toxicity or compound-related deaths but it resulted in dose-dependent, statistically significant decreases in body weight gain ($p<0.01$) and food consumption ($p<0.05$, $p<0.01$) for males and females in the medium and high dose groups as compared to controls. Body weight gains for males treated with Ziram at 300 and 1000 ppm were 82% and 67% of controls, respectively. Body weight gains for females treated with Ziram at 300 and 1000 ppm were 82% and 68% of controls, respectively.

Table 2. Acute toxicity data for ziram

Guideline No./Study Type		MRIDs	Results	Tox Category
870.1100	Acute Oral	41340401[a]	LD_{50} =320 mg/kg (M&F) LD_{50} = 381 mg/kg (M) LD_{50} = 267 mg/kg (F)	II
		42429301[b]	LD_{50} >2000 mg/kg (M&F)	III
		43701301[a]	LD_{50} =2068 mg/kg (M&F) LD_{50} = 2719 mg/kg (M) LD_{50} = 2060 mg/kg (F)	III
870.1200	Acute Dermal	41340402[a]	LD_{50} > 2000 mg/kg (M & F)	III
870.1300	Acute Inhalation	41442001[a]	LC_{50} =0.07 mg/L (M&F) LC_{50} =0.08 mg/L (M) LC_{50} = 0.06 mg/L (F)	II
870.2400	Primary Eye Irritation	41643001[a]	Severe irritation	I
		41454401[b]	Severe irritation	I
870.2500	Primary Skin Irritation	41643002[a]	Not a dermal irritant	IV
		41454602[b]	Not a dermal irritant	IV
870.2600	Dermal Sensitization	41643003[a]	Moderate dermal sensitizer; 30% sensitization rate	NA

a Technical
b 76% formulation

Food consumption for males treated with Ziram at 300 and 1000 ppm was decreased to 87% and 75% of controls, respectively. Although there were no statistically significant decreases in body weight, the percentage decreases were levels generally regarded as toxicologically significant, i.e., 9-12% and 8-11% for the 300 ppm males and females, respectively; 20-21% and 13-16% for the 1000 ppm males and females, respectively. Food consumption for females treated with Ziram at 300 ppm and 1000 ppm was decreased to 83% and 75% of controls, respectively. Food efficiency for males and females was slightly decreased for the medium and high dose groups as compared to controls. There were statistically significant increases in relative organ weights of brain for males treated with Ziram at 300 ppm ($p<0.05$) and 1000 ppm ($p<0.01$) and of brain and spleen for females ($p<0.01$, $p<0.05$) at 300 ppm and 1000 ppm ($p<0.01$ for both) that were not found in controls or in rats treated with Ziram at 100 ppm, but no concomitant histopathological findings. There were no biologically-relevant changes in hematological or chemistry parameters at any dose groups. There were microscopic pathological findings of localized areas of epithelial hyperplasia in the stomach (3 females and 1 male in the 1000 ppm and 1 female in the 300 ppm dose group vs. 0 in control groups and centrilobular necrosis in one liver lobe in 1 female each in the 300 and 1000 ppm groups vs. 0 in the control animals). No lesions were noted for the 100 ppm dose group. Based primarily on the decreases in body weight, body weight gain, food consumption and minimal histopathological changes in the liver (females) for the mid-dose group, and the lack of findings for the low-dose group, **the LOAEL was chosen as 300 ppm (M:21.4**

mg/kg/day; F: 24.2 mg/kg/day) and the NOAEL as 100 ppm (M: 7.4 mg/kg/day; F: 8.8 mg/kg/day).

The study is classified as acceptable/guideline and satisfies the guideline requirements for a subchronic feeding study (§82-1) in rats.

870.3150 90-Day Oral Toxicity - Dog

The chronic dog study presented later in section 4.5 (870.4100b) satisfies the data requirements for 870.3150.

870.3200 21/28-Day Dermal Toxicity - Rabbit

In a 21-day repeated dose dermal toxicity study (MRID 41297001), groups of 5 male and 5 female New Zealand white rabbits were treated with Ziram Technical (98.5%) in distilled water by dermal occlusion at doses of 0, 100, 300, or 1000 mg/kg/day for 6 hours/day for 21 days.

No mortality was observed, and there were no treatment-related dermal lesions. There were also no effects on organ weights, macroscopic pathology, or histopathology. Decreased bodyweight ($p<0.05$; 9-13%) was observed in high-dose females all three weeks of the study. Decreased food consumption ($p<0.05$; 34%) was observed in high-dose females during the first week of the study. Decreased lymphocyte counts ($p<0.05$) were observed in high-dose females; however, this effect is not considered toxicologically significant. Changes in clinical pathology parameters [increased GPT (ALT), GOT (AST), bilirubin and cholesterol] in the high-dose females indicated minimal hepatotoxicity.

Under the conditions of this study, the NOAEL for systemic toxicity in females for Ziram Technical was 300 mg/kg/day. The LOAEL for systemic effects was 1000 mg/kg/day based on decreased body weight and food consumption and clinical chemistry changes suggestive of minimal hepatotoxicity (increases in GPT, GOT, bilirubin and cholesterol). The NOAEL for males is greater than 1000 mg/kg/day; the LOAEL was not identified. The NOAEL for dermal effects in both sexes was equal to or greater than 1000 mg/kg/day; the LOAEL was not identified.

This study is classified as **acceptable (guideline)** and satisfies the guideline requirements for a 21-day dermal study (82-2) in rabbits.

870.3250 90-Day Dermal Toxicity

There was no 90-day dermal toxicity study. A data gap for a 90-day dermal study will be considered if there is intentional exposure.

870.4365 90-Day Inhalation Toxicity

A 28-day inhalation study has been identified as a data gap by the HIARC. A 90-day inhalation study is not required at this time.

4.3 Prenatal Developmental Toxicity

Adequacy of data base for Prenatal Developmental Toxicity: The data base for prenatal developmental toxicity is considered complete and no additional studies are required at this time. In the rat developmental study, there was no increased susceptibility of the fetus to the test substance; an increased incidence of thinning of the diaphragm was noted. Maternal toxicity in the rat included post-dosing salivation, reduced body weights, decreased food consumption, and increased water consumption. In the rabbit developmental study, there was no increased susceptibility of the fetus to the test substance. Increased resorptions/doe, increased postimplantation loss, and reductions in the number of live fetuses/doe were observed. Maternal toxicity in the rabbit consisted of decreased body weights, body weight gains, and food consumption.

870.3700a Prenatal Developmental Toxicity Study - Rat

Presumed pregnant Crl:CD® (SD) BR VAF/Plus rats, randomly assigned to one control and four treatment groups of 25 animals each, were administered Ziram by gavage at doses of 0, 1, 4, 16, or 64 mg/kg on gestation days (GD) 6-15 inclusive. Cesarean section examinations were performed on all surviving dams on GD 20, followed by external examination of all fetuses. Approximately one-half of each litter was examined for visceral anomalies and the remainder was fixed and stained for skeletal examinations. There were at least 22 pregnant animals per group.

All animals survived to terminal sacrifice on GD 20. Post-dosing salivation, generally during the last few days of dosing, was associated with treatment at 16 mg/kg in 2 of 25 animals and at 64 mg/kg in 9 of 25 animals. Generalized hair loss occurred in 2/25 control and 6/25 high-dose animals. No clinical signs were associated with treatment with 1 or 4 mg/kg/day. Significantly ($p \leq 0.05$) reduced body weights (approximately 92% of control) occurred in the 64 mg/kg/day-group as compared to controls during the treatment interval and continuing until sacrifice. The 16 mg/kg-group also had significantly ($p \leq 0.05$) reduced mean body weight (94%) as compared to the control group during the treatment period; however,

recovery occurred after treatment ended. Mean food consumption was significantly ($p \leq 0.01$) decreased in the 16 and 64 mg/kg- groups as compared to controls beginning with GD 6-7 during the treatment interval and continuing to GD 16-17 for the high-dose group. In contrast, water consumption was significantly ($p \leq 0.01$) increased in the 16 and 64 mg/kg groups during the treatment period as compared to the control group. Water intake continued to be greater ($p \leq 0.05$) than controls for these two groups after the treatment interval but returned to the control level on the last day of the study. **Therefore, the maternal toxicity LOAEL is 16 mg/kg/day based on decreased body weights, reduced food consumption, and salivation during the treatment interval and the maternal toxicity NOAEL is 4 mg/kg/day.**

Mean fetal body weights of the high-dose litters were significantly ($p \leq 0.01$) lower than controls (89%). There were no differences between treated and control groups for number of fetuses per litter, implantations per dam, number of resorptions per dam, or fetal sex ratios, and there were no dams with whole litter resorption. Overall, there was no significant difference or dose-related trend in the number of treated litters affected as compared to control when the incidences of external, visceral, and skeletal malformations/ variations were combined. The number of litters affected in the control, 1, 4, 16, and 64 mg/kg-groups was 13 of 23, 9 of 24, 8 of 22, 19 of 23, and 11 of 24, respectively. No treatment-related external or skeletal malformations/variations were seen in any fetuses from any group. However, there was a dose-related increase in the incidence of diaphragmatic lesions. The incidence of thinning of the diaphragm with protrusion of the liver occurred in 0/23, 0/24, 1/22, 4/23, and 6/24 ($p \leq 0.05$) litters in the 0, 1, 4, 16, and 64 mg/kg-groups, respectively. **Therefore, the developmental toxicity LOAEL is 16 mg/kg/day based on diaphragmatic thinning, and the developmental toxicity NOAEL is 4 mg/kg/day.**

Classification: **Acceptable/Guideline**

This study satisfies the guideline requirement for a developmental toxicity study (83-3) in rats.

870.3700b Prenatal Developmental Toxicity Study - Rabbit

In a developmental toxicity study (MRID 00161316), ziram technical (98% a.i.) in 1% aqueous methyl cellulose was administered by gavage to pregnant New Zealand White rabbits (16/dose) at concentrations of 0, 3, 7.5, or 15 mg/kg/day on GDs 7 through 19. Does were sacrificed on GD 28.

One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. These deaths were not considered to be the result of treatment due to the lack of a

dose-response relationship. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

At 15 mg/kg, decreased body weights were observed over GDs 0-28 (↓3-17%, $p \leq 0.01$ on GD 10 only). Additionally, for the overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced as compared to the control (treatment, ↓81%; study, ↓30%, not analyzed for statistical significance). Decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption were observed beginning at the GDs 7-10 interval (↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28).

At 7.5 mg/kg, decreased body weight gain was observed over GDs 7-19 (↓30%) and GDs 0-28 (↓19%). No other treatment-related maternal effects were noted at the mid-dose level.

No treatment-related findings were observed at gross necropsy of maternal animals.

The number of implantations/doe and percent male were similar between control and treated groups.

The maternal LOAEL is 7.5 mg/kg/day, based on decreased body weight gain. The maternal NOAEL is 3 mg/kg/day.

Reduced atrium/atria, a minor defect (variation), was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%). The HIARC did not considered this as an effect since there was no dose response, there were no statistically significant differences in the incidences at any dose, and the data on this parameter, which is a highly subjective observation, showed a wide-spread variation in the size of the atria (enlarged and reduced) among the control and the treatment groups.

At the high-dose level, increases (NS) as compared to the control were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted. Upon skeletal examination, absence of the interparietal bone, a major defect (malformation), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered equivocally treatment-related.

The developmental LOAEL is 15 mg/kg/day, based upon increased resorptions and post-implantation loss. The developmental NOAEL is 7.5 mg/kg/day.

This developmental toxicity study is classified **acceptable/guideline (§83-3[b])** and <u>does satisfy</u> the guideline requirement for a developmental toxicity study in the rabbit; it would be

helpful if historical control data (% fetal and % litter incidences) were provided for reduced atrium/atria and absence of the interparietal bone.

4.4 Reproductive Toxicity

Adequacy of data base for Reproductive Toxicity: The data base for reproductive toxicity is considered complete and no additional studies are required at this time. The two-generation rat reproduction study revealed decreased pup weights at birth and/or during lactation while parental toxicity resulted in decreased body weights, body weight gains, and food consumption. The study did not show evidence of increased offspring susceptibility.

870.3800 Reproduction and Fertility Effects - Rat

Ziram (97.8% a.i.) was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). Premating doses for the F_0 males were 5.3, 14.8, and 37.5 mg/kg, respectively and for the F_0 females were 6.1, 16.8, and 42.8 mg/kg, respectively. Premating doses for the F_1 males were 5.6, 16.7, and 42.7 mg/kg, respectively, and for the F_1 females were 6.3, 18.4, and 47.5 mg/kg, respectively. Each generation contained 30 animals/sex/dose which were given test or control diet for at least 10 weeks then mated within the same dose group. F_1 animals were weaned on the same diet as their parents. Sibling matings were avoided and at least 23 litters were produced in each generation. All animals were exposed to test material either in the diet or during lactation until sacrifice. The time course for the study was as follows: weeks 1-10, F_0 premating; weeks 11-18, F_0 breeding, gestation, and lactation; weeks 19-30, F_1 premating; week 39, end of study.

All F_0 and F_1 parental animals survived to scheduled necropsy. Generalized, clinical signs in the adult animals, such as hair loss and sores, were observed in the control and treated animals equally and there was no correlation with dose.

No treatment-related effects were seen in the 72 or 207 ppm groups of either generation as compared with controls. High-dose F_0 males initially had lower body weights (90-93%) than controls at weeks 1, 2 ($p \leq 0.01$), and 3 ($p \leq 0.05$) due to a significantly ($p \leq 0.01$) lower body weight gain (71%) during week 0-1. Throughout the remainder of the study, there were no significant differences in absolute body weights of the treated F_0 male groups as compared to controls. Food consumption by the high-dose F_0 males was significantly ($p \leq 0.01$) less than controls for the first 4 weeks of the study and at weeks 8-9, 9-10 ($p \leq 0.05$), and 10-11. Body weights of the high-dose F_0 females were significantly ($p \leq 0.01$) less than the controls for the entire premating period (92-94%). However, body weight gains were significantly less than controls only during week 0-1 (44%; $p \leq 0.01$), week 1-2 (76%; $p \leq 0.05$), and week 6-7 (67%; $p \leq 0.01$). High-dose F_0 females ate significantly ($p \leq 0.01$) less than the controls throughout the entire premating period.

High-dose F_1 males had significantly ($p \leq 0.01$) lower body weights (97-90%) as compared to controls throughout the entire premating period and continuing until study termination. Body weight gains in the high-dose males were significantly less than the controls during study weeks 18-19, 20-21 (83%; $p \leq 0.01$), and 21-22 (90%; $p \leq 0.05$) of the premating period. Food consumption was significantly less than the controls for the high-dose F_1 males ($p \leq 0.01$) throughout the entire premating period. Absolute body weights of the high-dose F_1 females were significantly lower than the controls for the entire premating period (89-92%; study weeks 19-23, $p \leq 0.05$; weeks 24-30, $p \leq 0.01$); significantly lower body weight gains (67-87%) occurred only during study weeks 18-19 ($p \leq 0.05$), 23-24, and 24-25 ($p \leq 0.01$). Food consumption by the high-dose F_1 females was also significantly less than the controls throughout premating ($p \leq 0.01$; weeks 21-22 and 28-29, $p \leq 0.05$).

There were no treatment-related gross- or histological abnormalities observed in either generation. Differences in absolute and relative organ weights of the high-dose male and female F_0 and F_1 groups as compared to controls are consistent with reduced body weights of these animals.

Therefore, the systemic toxicity LOAEL is 540 ppm (37.5 mg/kg/day) based on reduced body weights, body weight gains, and decreased food consumption by F_0 and F_1 males and females. The systemic toxicity NOAEL is 207 ppm (14.8 mg/kg/day).

High-dose F_0 animals had significantly ($p \leq 0.01$) lower body weights as compared to controls throughout gestation and until day 14 of lactation; body weight gains were significantly ($p \leq 0.05$) less than controls during the day 10-14 interval of gestation. Some recovery was apparent in the high-dose F_0 females with body weight gains significantly ($p \leq 0.01$) greater than the controls during lactation days 14-21; this resulted in overall body weight gains during lactation significantly greater than the controls. On gestation day 20 and lactation day 21, body weights of the high-dose F_0 animals were 90% and 98% of the control level. High-dose F_0 females also had significantly ($p \leq 0.01$) lower food consumption as compared to controls throughout gestation and during days 4-7 ($p \leq 0.05$) and 7-14 of lactation. The high-dose F_1 females had significantly lower body weights throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose F_1 animals were 89% and 93% of the control level. Food consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls by the high-dose group throughout gestation and lactation.

No dose- or treatment-related effects were noted on the reproductive performance of adults from either generation. F_1 pups from high-dose group dams had consistently lower body weights than controls beginning at day 4 precull with significance (92%; $p \leq 0.01$) reached on day 14. High-dose F_2 pups also had lower body weights than the controls throughout lactation

with significance reached on days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$).

Therefore, the LOAEL for offspring toxicity is 540 ppm (42.8 mg/kg/day) based on reduced pup body weights at birth in F_2 pups and during lactation in both F_1 and F_2 pups. The corresponding NOAEL for offspring toxicity is 207 ppm (16.8 mg/kg/day).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4) in rats.

4.5 Chronic Toxicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete and no additional studies are required at this time. In the chronic toxicity/oncogenicity rat study, erythrocytes, hemoglobin, hematocrit, calcium, total protein, albumin, calcium, and alanine aminotransferase were decreased (in the females). Gross and/or microscopic pathological findings were noted in the stomach, skeletal muscle, adrenal glands, spleen, liver, thyroid, spinal cord, and sciatic nerve. In addition, body weight gains and food consumption were decreased. In the dog study, signs of toxicity included a convulsive episode in one animal, decreased body weight gains in the females, and histological findings in the male livers.

870.4100a Chronic Toxicity – Rat

Male and female CD(SD)BR rats, 50/sex/dose in the main group, 20/sex/dose in the satellite group were treated with Ziram (98.7%, Lot# 8331 AA) at 0, 60, 180, and 540 ppm for 104 weeks, MRID No. 43404201. These doses corresponded to achieved intakes of 0, 2.5, 7.7, and 23.7 mg/kg/day for males in the main group and 0, 3.4, 10.2, 34.6 mg/kg/day for females in the main group.

There was no excess mortality in any of the treated groups relative to controls. Group mean body weight gains were decreased for males (86% of control, $p<0.01$ and females (74% of control, $p<0.01$) in the high dose group (540 ppm). Food consumption was decreased compared to controls for males (540 ppm: 91%, $p<0.01$) and females (180 ppm: 92%, $p<0.05$; 540 ppm: 94%, $p<0.05$). Hematology parameters (RBC, HGB, and PCV) were decreased relative to controls for females in the 540 ppm (weeks 26-104, $p<0.05$, $p<0.01$) and 180 ppm (weeks 26-52, $p<0.05$, $p<0.01$)) dose groups. There were statistically significant decreases ($p<0.05$, $p<0.01$) in clinical chemistry parameters (calcium, total protein, albumin, calcium and SGPT) during weeks 13-52 for females. For males (540 ppm, week 104) organ weight for the adrenals was decreased (absolute, 59% of control, $p<0.01$; relative, 67% of control, $p<0.05$). There were macroscopic pathological findings (not statistically significant) for animals in the 180 and 540 ppm dose groups for the stomach and skeletal muscle (males and females), and the adrenals (females only). There were microscopic pathological findings for males and females in the 180 and 540 ppm dose groups for spleen ($p<0.01$), liver ($p<0.01$,

p<0.05), stomach (p<0.05, p<0.01), thyroid (p<0.01, p<0.05), skeletal muscle (p<0.01), spinal cord (males only, p<0.05), sciatic nerve (females only, p<0.01), and adrenal cortex (p<0.05, p<0.01). As there were histopathological findings for males in the 60 ppm dose group for spleen (p<0.01), stomach (p<0.01, p<0.05), skeletal muscle (p<0.05), and adrenal cortex (p<0.05), a NOAEL for males could not be identified. For females, there was an increase in prominent ultimobranchial cysts in the thyroid in all dose groups (Controls: 3/50; 60 ppm: 12/50, p<0.05; 180 ppm: 22/50, p<0.01;, 540 ppm: 27/50, p<0.01), precluding the identification of a NOAEL for females. **The NOAEL could not be identified for either males or females, due to histopathological findings for animals in the low dose group (60 ppm).**

Carcinogenic potential was evidenced by the finding of treatment-related tumors (benign hemangioma) in mesenteric lymph nodes (5/50, p<0.05) and in spleen (1/50) in males in the 540 ppm dose group. There were no treatment-related tumors identified in males in the 180 or 60 ppm dose groups, or in females in any dose group. There were no treatment-related malignant tumors in either sex. The dosing is adequate. Treatment of males with Ziram for 104 weeks at the MTD resulted in neoplastic changes.

This study is classified as Acceptable and satisfies the guideline requirements for a chronic/oncogenicity study (§83-5). This study did not establish a NOAEL.

870.4100b Chronic Toxicity - Dog870.4100b Chronic Toxicity - Dog

In a chronic feeding study (MRID No. 42823901), ziram (98.5%; Lot No. 8331 AA) was administered for 52 weeks in the diet to four male and four female beagle dogs per dose at concentrations of 0, 50, 185, and 700 ppm (700 ppm dose reduced to 500 ppm at day 3 of week 12), equivalent to doses of 0, 1.6, 6.6, 17.4 mg/kg/day for males and 1.9, 6.7, and 20.6 mg/kg/day for females, respectively.

There was a treatment-related convulsive episode at week 11 for a female in the 700/500 ppm dose group that required the animal to be euthanitized. In addition to the convulsive episode, the findings for the 700/500 ppm dose group include:1) decreased body weight gain (↓81%) in females over the treatment period and 2) histologic findings for livers (aggregates of Kupffer cells and macrophages, increased foci of degenerate hepatocytes, infiltration of inflammatory cells around central veins and branches of the hepatic vein and portal areas, and increased centrilobular fibrocytes in males). The findings for the 185 ppm dose group include decreased body weight gain (↓69%) in females during the treatment period.

The NOAEL is 50 ppm based on the lack of significant toxicological effects. The LOAEL is 185 ppm based on decreased body weight gain in females.

This study is classified as **acceptable/guideline (§83-1)** and satisfies the requirements for a chronic feeding study in beagle dogs.

4.6 Carcinogenicity

Adequacy of data base for Carcinogenicity: The data base for carcinogenicity is considered complete and no additional studies are required at this time. In the chronic toxicity/oncogenicity rat study, evidence of carcinogenic potential in the males included benign hemangioma in the mesenteric lymph nodes and the spleens as well as combined hemangiomas of the mesenteric lymph node and spleen. The NTP 2-year carcinogenicity study in rats concluded that the test substance was carcinogenic in males, causing increased incidences of C-cell carcinomas of the thyroid gland. No treatment-related increases in tumor incidences were noted in the mouse oncogenicity study. The NTP 2-year carcinogenicity study in mice resulted in increased incidences of alveolar/bronchiolar adenomas or carcinomas in the females.

870.4200a Carcinogenicity Study - Rat

The chronic/oncogenicity study presented above in section 4.5 (870.4100a) satisfies the data requirements for 870.4200a.

National Toxicology Program Two-year Carcinogenicity Study with ziram in F344/N rats

Executive Summary: In a 2-year carcinogenicity feeding study, Ziram (89% pure, with 6.5% thiram) was administered in the diet to 50 male and 50 female F344/N rats per group at 0, 300, or 600 ppm. The doses corresponded to overall mean doses of about 0,11, or 22 mg/kg/day for males and to 0, 13, or 26 mg/kg/day for females.

There were no treatment-related effects on mortality, clinical signs, body weight, or food consumption, and minimal non-neoplastic histopathology. C-cell hyperplasia in the thyroid gland was noted in males in the control and all dose groups, but did not appear to be dose related (control, 7/50, 14%; 300 ppm, 12/49, 24%; 600 ppm, 11/49, 22%). No treatment-related effects on C-cell histopathology were noted in females.

NTP concluded that ziram was carcinogenic for male F344/N rats, causing increased incidences of C-cell carcinomas of the thyroid gland, but not carcinogenic for female F344/N rats.

Citation: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (Cas No. 137-30-4) in F344/N Rats and B6C3F_1 Mice (Feed Study)(NTP Technical report series No. 238), Research Triangle Park, NC.

Combined Chronic Toxicity/Carcinogenicity Study- CD(SD)BR rats

Male and female CD(SD)BR rats, 50/sex/dose in the main group, 20/sex/dose in the satellite group were treated with Ziram (98.7%, Lot# 8331 AA) at 0, 60, 180 or 540 ppm for 104 weeks. These doses corresponded to achieved intakes of 0, 2.5, 7.7 or 23.7 mg/kg/day for males in the main group and 0, 3.4, 10.2 or 34.6 mg/kg/day for females in the main group.

The dietary administration of ziram at 540 ppm resulted in a treatment-related increased incidence of hemangiomas of mesenteric lymph nodes (5/49 or 10%) and spleen (1/49 or 2%), as well as combined hemangiomas of the mesenteric lymph node and spleen (6/49 or 12%) in male rats. These tumors rarely metastasize. There were no hemangiomas in the control group. Incidences of hemangiomas in historical controls ranged from 0 to 4% (mean = 1%) in lymph nodes and 0% in the spleen. The statistical evaluation of hemangiomas in male rats revealed significant increasing trends at $p < 0.01$ and significant differences in the pair-wise comparisons of the 540 ppm group with the controls at $p < 0.05$ for mesenteric lymph node hemangiomas and for lymph node and spleen hemangiomas combined. No hemangiosarcomas were reported in any dose group and no treatment-related tumors were identified in males in the 60 or 180 ppm groups or in females at any dosage level.

In males and females from the 180 and 540 ppm dose groups, there were findings of hemosiderosis in the spleen and sinusoidal cells of the liver, bile duct hyperplasia, hyperplasia of the non-glandular epithelium of the stomach, subepithelial edema and ulcerations in the stomach, prominent ultimobranchial cysts in the thyroid, and axonal degeneration (minimal) in the spinal cord (males), and axonal degeneration in the sciatic nerve (males, not statistically significant; females, $p<0.01$). In addition, the degeneration was of greater severity in treated animals than in controls and generally increased in severity with increasing dose of Ziram. There were findings for males only in the 180 and/or 540 ppm dose groups, including adipose replacement of pancreatic tissue, C-cell hyperplasia in the thyroid, hyperplasia in the parathyroids, and hypertrophy with vacuolation in the adrenal cortex.

Citation: Lindsey A. J. Powell, Sarah M. Bottomley, David Crook, Richard L. Gregson, John M. Offer, William A. Gibson, Alan Anderson (1994) Combined chronic toxicity and oncogenicity of Ziram (Technical) administered in the diet to rats. Huntingdon Research Centre Ltd. Laboratory report number: ZIR 9/942098 September 27, 1994. MRID 43404201. Unpublished.

870.4200b Carcinogenicity (feeding) - Crl: CD-1 (ICR) BR Mouse

In an 80-week oncogenicity feeding study (MRID No. 43373701), Ziram (98.7%, Lot No. 8331 AA) was administered in the diet to 50 male and 50 female Crl: CD-1 (ICR) BR mice per group at 0, 29, 75, 225, or 675 ppm. The doses corresponded to overall mean doses of about 0, 3, 9, 27, and 82 mg/kg/day for males; and to 0, 4, 11, 33, and 95 mg/kg/day for females.

Significantly decreased mean weight gain was seen in males at 225 ppm (77% of control) and at 675 ppm (56% of control). In females in the 225 ppm, weight gain was decreased to about 94% of the control group. The mean weight gain in females was significantly decreased at 675 ppm compared to control values (80% of control). Dose-related decreases in mean absolute brain weights were seen in both sexes, but, although numerically greater in females, were statistically significant only in males at 225 and 675 ppm. The incidence of centrilobular hepatocyte enlargement was increased in all treated animals. The incidence reached maximums of about 50% in males and 38% in females at 75 and 225 ppm then dropped at the high dose to 39% in males and 14% in females. These effects seem to indicate an adaptive response at all doses since there was no effect on liver weight, no dose-related effect on the gradation of the pathology (minimal at all doses), and no necrosis seen even at the high dose. Significant increases in the incidences of urinary bladder epithelial cell hyperplasia were seen in males at 225 and 675 ppm (39 and 70%, respectively, in terminal animals compared to 18% in controls), and in females at 675 ppm (20% in terminal animals compared to 0 in controls). Urinary bladder epithelial hypertrophy was significantly increased in terminal females at 675 ppm (38% compared to 8% in controls).

The NOAEL is 75 ppm. The LOAEL is 225 ppm based on decreased absolute brain weights in both sexes and significantly increased incidence of urinary bladder epithelial hyperplasia and decreased body weight gain in males.

There were no treatment-related increases in tumor incidences.

This study is Acceptable/Guideline and satisfies the guideline requirements for a oncogenicity feeding study in mice (83-2).

870.4200b Carcinogenicity (feeding) - B6C3F$_1$ Mouse

National Toxicology Program Two-year Carcinogenicity Study with ziram in B6C3F$_1$ mice

In a 2-year carcinogenicity feeding study, Ziram (89% pure, with 6.5% thiram) was administered in the diet to 50 male and 50 female B6C3F$_1$ mice per group at 0, 600, or 1200 ppm. The doses corresponded to overall mean doses of about 22 or 196 mg/kg/day for males and to 0, 131, or 248 mg/kg/day for females.

No treatment-related effects on mortality or clinical signs were noted. There were treatment-related decreases in mean body weight gain in males (both doses: 10-25% decrease compared to controls) and in females (1200 ppm: 13-23% decrease after day 80 compared to controls), decreases in food consumption at 1200 ppm in males (78% of control) and in females (85% of control), and histopathology findings.

Alveolar epithelium hyperplasia in the lungs was noted in females in the control group and both dose groups and was dose-related (control, 2/50, 4%; 600 ppm, 4/49, 8%; 1200 ppm, 10/50, 20%). Alveolar epithelium hyperplasia in the lungs was not noted in males at any dosage level. Pulmonary adenomatous hyperplasia was noted in control and dosed males (control, 15/49, 31%; 600 ppm, 19/50, 38%; 1200 ppm, 16/49, 33%) and in control and dosed females (control, 18/50, 36%; 600 ppm, 27/49, 55%; 1200 ppm, 26/50, 52%). This particular histopathological finding is consistent with chronic Sendai virus infection which was confirmed by serology performed on untreated animals housed in the same room and from the same shipment. Six of the 26 1200-ppm group females with the adenomatous hyperplasia had pulmonary tumors, whereas four of the 24 1200-ppm group females without pulmonary adenomatous hyperplasia had pulmonary tumors also. One of 27 600-ppm group females with adenomatous hyperplasia had a pulmonary tumor. Cystic follicles in the thyroid occurred at increased incidences in females at 1200 ppm (controls, 0/47; 1200 ppm, 21/48, 44%). Lymphoid hyperplasia was seen at increased incidences at 600 and 1200 ppm in females (controls, 0/50; 600 ppm, 2/50, 2%; 1200 ppm, 7/50, 14%).

NTP concluded that oral administration of ziram to female $B6C3F_1$ mice resulted in increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas. The interpretation of this increase in lung tumors, however, was complicated by an intercurrent Sendai virus infection.

Citation: U.S. National Toxicology Program (1983) Carcinogenesis Bioassay of Ziram (Cas No. 137-30-4) in F344/N Rats and $B6C3F_1$ Mice (Feed Study)(NTP Technical report series No. 238), Research Triangle Park, NC.

870.4300 Chronic/Oncogenicity

The chronic/oncogenicity study presented above in section 4.5 (870.4100a) satisfies the data requirements for 870.4300.

4.7 Mutagenicity

Adequacy of data base for Mutagenicity: The data base for mutagenicity is considered incomplete. The CARC has recommended that a dominant lethal assay be conducted by the registrant to address a possible concern for heritable effects. Ziram is primarily a bacterial mutagen with the capacity to bind to macromolecules (i.e., cholinesterase). Although results of mammalian cell assays were in conflict, the preponderance of data from the cytogenetic studies favor a positive response. Based on the direct mutagenic effect on base-pair substitution strains of *S. typhimurium* and *E. coli* and the evidence of clastogenicity in mammalian cells, the Committee determined that there is sufficient evidence for a mutagenic concern.

Gene Mutation

870.5265- Salmonella typhimurium/mammalian activation gene mutation assay MRID 00147462 Acceptable	*S. typhimurium* was exposed to ziram at concentrations of 10-333.3 µg/plate ±S9. The test article was positive for gene mutation induction in strain TA100 with and without S9 activation.
870.5265-Salmonella typhimurium/mammalian activation gene mutation assay MRID 41642901 Acceptable	*S. typhimurium* was exposed to ziram at concentrations of 0.5-50 µg/plate ±S9. Additionally, the TA100 strain was evaluated at concentrations of 15-150 µg/plate in the presence of 5-20% S9 fraction and at 25-100 µg/plate in the presence of 20-30% S9 fraction. The test article was mutagenic when tested above 50 µg/plate in the presence of the S9 fraction. A dose-related genotoxic response in the TA100 strain (+S9) was observed with increases in mutant colonies at 50 µg/plate and above.
870.5265-Salmonella typhimurium/mammalian microsome gene mutation assay Haworth *et al.*, 1983 Acceptable	Results were positive for strain TA100 at 10-333 µg/plate without and with rat S9 and at 33-333 µg/plate with hamster S9. Increases of approximately 2-fold were also seen for strain TA 1535 at 100 µg/plate with rat liver S9 or at 33-333 µg/plate with hamster liver S9.
870.5300-*In vitro* mammalian cell forward gene mutation assay in mouse lymphoma L5178Y cells McGregor et al., 1988 Acceptable	The test article was positive for the induction of gene mutations at 0.1-1.8 µg/plate. The test was only performed in the absence of S9.

Cytogenetics

870.5375-*In vitro* mammalian cytogenetics assay MRID 41287802 Acceptable	Chinese hamster ovary (CHO) cells were exposed to ziram at concentrations ranging from 0.8 to 40,000 ng/mL without S9 activation and from 5 to 40,000 ng/mL with S9 activation. There was no evidence of structural chromosomal aberrations over background.
870.5375-*In vitro* mammalian cell cytogenetic assay in CHO cells Gulati, 1989 Acceptable	produced increases in structural chromosome aberrations at 0.025 and 0.05 ug/mL without S9 activation and 1.5 and 1.75 ug/mL with S9 activation.
870.5395-870.5375-*In vitro* mammalian cell cytogenetic assay in mice, MRID 44989301 Unacceptable/upgradable	The test article when tested up to 675 ppm (≈101.25 mg/kg/day) did not induce a significant increase in the incidence of MPCEs or MNCEs compared to vehicle controls. Testing at the maximum tolerated dose (MTD) was not conducted and a positive control was not used to confirm assay sensitivity. Also the data from the dose formulation analysis were not presented.

Other Genotoxicity

870.5550, Unscheduled DNA synthesis MRID 41287801 Acceptable	Primary rat hepatocyte cultures were exposed to ziram at 12 concentrations ranging from 0.316 to 100,000 ng/mL for 19 and 20 hours. There was no evidence that unscheduled DNA synthesis was induced.

4.8 Neurotoxicity

Adequacy of data base for Neurotoxicity: The data base for neurotoxicity is considered complete and no additional studies are required at this time. In the acute neurotoxicity screening battery in rats, death, clinical signs of neurotoxicity, changes in the FOB (ataxia, salivation, lacrimation, impaired gait, abnormal posture), decreased motor activity, and decreased absolute brain weights were observed. In the subchronic neurotoxicity screening battery, inhibition of brain neurotoxic esterase activity and decreased brain cholinesterase activity were noted as well as decreased body weights, body weight gains, and food consumption. In the developmental neurotoxicity study, increased motor activity, decreased startle response, and decreased body weights and body weight gains were observed in the offspring. Maternal toxicity included decreased body weights, body weight gains, and food consumption. Other studies where evidence of neurotoxicity was presented included the chronic dog (MRID 42823901; axonal degeneration of the sciatic nerve and a convulsive episode in one animal) and mouse carcinogenicity (MRID 43373701; decreases in absolute brain weight) studies.

870.6100 Delayed Neurotoxicity Study - Hen

These studies are not required at this time.

870.6200a Acute Neurotoxicity Screening Battery

In an acute neurotoxicity study (MRID 43362801), male and female Sprague-Dawley Crl:CD BR® rats received a single gavage dose of 0, 15, 300, or 600 mg/kg of Ziram (tech., 97.8% a.i.) in corn oil (7.5 mL/kg). The 0, 15, and 300 mg/kg groups consisted of 12 animals/sex, the 600 mg/kg group consisted of 16 animals/sex. Functional observational battery tests (FOB) and motor activity were recorded for all animals. FOB and motor activity evaluations were conducted pretreatment, at the time of peak effect (4 hours post-dosing), and on days 7 and 14. At necropsy, brain weights and dimensions were determined for all animals. Five animals/sex were selected for neuropathological evaluation in the control and 600 mg/kg groups.

Four males and three females in the high-dose group died on day 1; three other high-dose females died on days 2, 4, or 5 and one mid-dose female died on day 2. The cause of these deaths is unknown. Gross observations at necropsy revealed white contents of stomach/intestines (probably from corn oil), stomach distention, and in two high-dose

females, emaciation. There were no findings consistent with trauma induced by gavage error. The two severely affected mid-dose males (Nos. 15355 and 15387) that survived the 2-week observation period exhibited cyanosis and hypothermia. However, neither cyanosis nor hypothermia were reported in the animals that died on study.

No effects on body weight were apparent in the low-dose group and transient effects were seen in high-dose males. In the mid-dose males, the mean body weights were significantly ($p<0.01$) lower on days 7 (12%) and 14 (16%) for mid-dose males compared with the control group means. The decreased body weights in the mid-dose group on day 14 were attributed to two males (Nos. 15355 and 15387), with body weights of 159 g and 161 g, respectively, compared with a control group mean of 321 g. Body weights of females were not affected; however, body weight gain was transiently reduced during days 0-7 in both males and females at mid and high-dose.

The most significant and biologically relevant findings of treatment were clinical signs of toxicity and effects observed during FOB and motor activity tests. Although both sexes in the mid- and high-dose groups were affected, several of the findings were limited to or occurred most frequently in two mid-dose males (Nos. 15355 and 15387). Clinical signs were generally seen in the first week of the study (but persisted to day 15 in the two mid-dose males) and included dose-related increased incidences of gait alterations, abnormal respiration, abnormal excreta, and distended abdomen. Cyanosis and enophthalmus were limited to two mid-dose males (No. 15355 and 15387) and were seen on day 8 or later on three occasions. Rales observed on one occasion in one low-dose male cannot be clearly attributed to treatment with the test material.

In the FOB evaluations, all six of the functional domains were affected in the mid- and high-dose groups. In general, the responses occurred approximately 4 hours after dosing and were transient in nature (none persisted to day 7). Notable effects on day 0 included altered posture, palpebral closure (eye lid slightly drooping to shut), altered feces consistency, slight lacrimation, slight to severe salivation, red/crusty deposits around nose and mouth, impaired mobility and altered gait, and decreased body temperature. Impaired gait and ataxia were also noted in males at 15 mg/kg on day 0. During the FOB on day 14, several findings were noted in the two most severely affected mid-dose males (altered posture, altered palpebral closure, enophthalmus, impaired mobility, absent startle response and hindlimb extension). It should be pointed out that some findings in these two animals (gasping, mucous membrane change and color, impaired righting reflex) were not even observed on day 7 or day 0, or in animals in the high-dose group. A low incidence of effects on FOB parameters was seen in the low-dose group. These effects (affecting neuromuscular and CNS activity in 1-2 animals) were minimal and cannot be attributed unequivocally to treatment with the test material because of the subjectivity of the endpoints.

Significantly ($p<0.05$) decreased motor activity was seen in mid- and high-dose males and females. Total motor activity and ambulatory activity counts were reduced by as much as 82-87% and 76-87%, respectively, compared with controls. However, complete recovery was

observed by day 7 in mid-dose males and females and in high-dose females; high-dose males recovered fully by study day 14. Even though the mean counts were not affected in the mid-dose males on day 14, the total motor activity and ambulatory activity counts for males (Nos. 15355 and 15387) were lower than the respective controls and lower than their day 7 values.

There was a dose-related decrease in absolute brain weights which was statistically significant at 300 and 600 mg/kg. No treatment-related effects on brain dimensions were noted. No treatment-related lesions were observed in central or peripheral nervous system tissues examined from the control or high-dose group.

The LOAEL is 15 mg/kg, based on ataxia and slight impairment of gait in males. No NOAEL was determined.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for an acute neurotoxicity study (81-8) in rats.

870.6200b Subchronic Neurotoxicity Screening Battery

In a subchronic oral neurotoxicity study (MRID 43413701), 10 Sprague-Dawley Crl:CD®BR rats/sex/dose group received Ziram (tech., 97.87% a.i.) in the diet at concentrations of 0, 72, 207, or 540 ppm for 13 weeks. The average consumption of test material was 5, 14, or 34 mg/kg/day (males) and 6, 16, or 40 mg/kg/day (females). Functional observational battery (FOB) and motor activity tests were conducted on all animals during weeks 3, 7, and 12. In each group, 5 animals/sex were allocated to cholinesterase/neurotoxic esterase evaluations and 5 animals/sex to neurohistopathologic evaluations.

At 540 ppm, the mean weekly body weights in males and females were 7% to 11% lower compared with controls beginning at week 1 and throughout the study period. The cumulative body weight gains (weeks 0 to 13) in males and females, respectively, were 18% and 32% lower than control values due in part to reduced food consumption, particularly during the initial study week (31% and 24% of controls for males and females, respectively). **The LOAEL for systemic toxicity is 540 ppm (34 mg/kg/day in males, 40 mg/kg/day in females) based on decreased body weights and body weight gains; the corresponding NOAEL is 207 ppm (14 mg/kg/day in males, 16 mg/kg/day in females).**

At 13 weeks, statistically significant brain inhibition of brain neurotoxic esterase activity was observed at 540 ppm compared with controls (-47%, males and -38%, females). Decreased brain cholinesterase activity was seen in males at 540 ppm (16%) and in females at 207 ppm (15%) and at 540 ppm (23%). No treatment-related effects were observed in the FOB, motor activity tests or microscopic examinations. **The LOAEL for brain cholinesterase inhibition was 540 and 207 ppm in males and females respectively. In addition, inhibition of brain neurotoxic esterase activity was noted in both sexes at 540 ppm. The NOAEL was 207 and 72 ppm in males and females, respectively.**

This study is classified as **Acceptable/Guideline** because it was generally well conducted and satisfies all guidelines requirements for a subchronic neurotoxicity study in rats (82-7).

870.6300 Developmental Neurotoxicity Study

Ziram (97.8% a.i.) was evaluated for developmental neurotoxicity during the conduct of a two-generation reproduction study. Test article was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). These concentrations resulted in F_1, maternal doses of 5, 13, and 32 mg/kg/day, respectively, during gestation and 11, 30, and 79 mg/kg/day, respectively, during lactation. The developmental neurotoxicity of ziram was evaluated in the F_2 offspring. Behavioral alterations, motor activity measures, auditory startle response, learning and memory, and the age of sexual maturation (vaginal perforation and balanopreputial separation) were examined. Brain weights and dimensions were recorded, and gross and histopathological evaluation of the nervous system tissue was conducted.

No treatment-related maternal or offspring toxicity was observed in the 72 or 207 ppm groups as compared with controls.

All F_1 dams survived until scheduled sacrifice and there were no treatment-related clinical signs of toxicity or neurobehavioral alterations. The high-dose F_1, females had significantly lower body weights throughout gestation ($p \leq 0.05$ or 0.01) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose F_1, animals were 89% and 93%, respectively of the control level. Food consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls in the high-dose group throughout gestation and lactation. At necropsy, there were no treatment-related gross- or histopathological abnormalities observed in the dams, and differences in absolute and relative organ weights of the high-dose group as compared to controls were consistent with reduced body weights of these animals.

High-dose F_2 pups also had lower body weights than the controls throughout lactation, with significance reached on postnatal days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$). Mean body weights of the high-dose F_2 males and females were also statistically significantly ($p \leq 0.05$ or 0.01) less than the controls throughout the postweaning period. However, final (postnatal day 70) body weights of F_2 males and females were 93 and 96%, respectively, of the control values. Overall body weight gain of the high-dose males was 94% of the controls while overall weight gain of the high-dose females was 99% of the control value. The age of sexual maturation for F_2 pups was not affected by treatment.

No clinical signs of neurotoxicity were observed in the F_2 offspring during daily cageside observations or at detailed physical examinations. Motor activity (total and/or ambulatory counts) was increased at all treatment levels, often 2 to 3-fold greater than control and in a dose-related manner, in pups of both sexes. At the low dose, these increases are apparent beginning at postnatal day 17 and continuing through postnatal day 21, while at the mid and high doses, they initiate at postnatal day 13 and continue through both days 17 and 21. Motor activity counts for postnatal day 60 were similar for control and treated rats of both sexes. Mean peak startle response was decreased (approximately 30% from control) in an apparently dose and treatment-related manner in high-dose pups of both sexes at postnatal day 22; this finding was not observed on postnatal day 60. Mean latency to peak response, response duration, and average response values appeared to be unaffected in treated animals as compared with controls on postnatal days 22 and 60. Learning and memory evaluations (in a water T-maze) at postnatal days 11 and 70 were similar for control and treated offspring. Brain weights (whole and regional) and dimensions (length and width) were not affected by treatment at postnatal days 11 or 70. Qualitative histopathological evaluation of the nervous system tissues did not reveal any treatment-related findings.

The maternal LOAEL is 540 ppm (32 mg/kg/day) based on reduced body weights and/or body weight gains, and decreased food consumption during gestation and lactation. The maternal NOAEL is 207 ppm (13 mg/kg/day).

The offspring LOAEL is 72 ppm (5 mg/kg/day) based on increased motor activity on postnatal days 17 and 21 for both sexes. The offspring NOAEL is <72 ppm (5 mg/kg/day).

Although this study contains useful information regarding the developmental neurotoxic potential of ziram, it is **classified** as **Guideline Unacceptable** (§83-6; OPPTS 870.6300) due to the following major deficiencies: 1) Neurobehavioral data (motor activity, startle response, and cognitive function) were not presented as percent change from control or analyzed statistically. 2) Simple morphometric analysis of representative locations within the neocortex, hippocampus, and cerebellum was not performed for F_2 offspring during histopathological examination of the brain at postnatal days 11 and 70. This study can be upgraded upon the submission and review of acceptable statistical analysis and morphometric data.

4.9 Metabolism

Adequacy of data base for metabolism: The data base for metabolism is considered incomplete; a metabolite identification study is requested. In a core-supplementary rat metabolism study, orally administered ziram was rapidly absorbed and excreted via the urine and expired air, and significant amounts were excreted in the feces. Small amounts of test agent were widely distributed in the body. There were no significant quantitative or temporal differences between males and females in excretion. No metabolite identification study was

performed. In an unacceptable rat dermal absorption study, tissue distribution and excretion data suggested minimal dermal absorption.

870.7485 Metabolism - Rat

Groups of 15 male and 15 female rats were administered Ziram/^{14}C-Ziram by gavage at doses of 15 mg/kg (Group 2, single low dose), 15 mg/kg/day for 14 days followed by a single dose of radiolabeled Ziram (Group 3), or 352 mg/kg (Group 4, single high dose). Controls (Group 1) received only the methylcellulose vehicle. Radioactivity excreted in the urine and feces was monitored for 168 hours (single low dose, multiple low dose, and single high-dose), and expired air for all three dose groups was monitored for 96 hours. Additionally, radioactivity in tissues and carcass were measured (MRID 42391001).

Clinical signs were limited to excessive salivation, lacrimation, rough hair coat, and white matter in the feces for several animals in dose Group 2 and 3. Overall recovery of administered radioactivity ranged from 78.9% to 92.4%. ^{14}C-Ziram derived radioactivity was excreted in the expired air, feces, and urine. There were no significant quantitative or temporal differences in excretion of radioactivity between males and females. For all three treatment groups, excretion of ^{14}C-Ziram derived radioactivity (average for both sexes) was greatest in expired air (37%, 41%, and 50% for Groups 2, 3, and 4, respectively), and was associated with both CO_2 and volatile fractions. Urinary excretion accounted for 17 to 35% of the administered radioactivity and was slightly greater in the multiple low-dose group. Fecal excretion accounted for 9 to 18% of the administered radioactivity and was similar for all dose groups. Percent of radioactivity administered was low in tissues (<1%) and carcasses (≤1%) in all dose groups. Time-course data for excretion of ^{14}C-Ziram indicated rapid excretion via expired air in both low-dose groups (<24 hours) and in <48 hours in the high-dose group. Urinary and fecal excretion was nearly complete within 72 hours for both low-dose groups, but appeared to be multiphasic in the high-dose group with excretion peaks at 0-8 hours, 24-72 hours, and at 96 hours.

Orally administered Ziram appears to be rapidly absorbed and excreted via the urine and expired air, and significant amounts are excreted in the feces. Small amounts are widely distributed in the body.

Classification: Unacceptable/Guideline

This study satisfies, in part, the guideline requirements for a metabolism study (85-1) in rats. This study was intended to provide only information on the absorption, distribution and excretion of ^{14}C-Ziram; however, it did not identify the metabolites excreted.

870.7600 Dermal Absorption - Rat

No acceptable dermal absorption study was provided; however, the 21-day dermal rabbit presented above in section 4.2 (870.3200) and the rabbit oral developmental presented above in section 4.3 (870.3700b) together satisfy the data requirements for 870.7600.

5.0 TOXICITY ENDPOINT SELECTION (HIARC, 2001)

5.1 Dietary Exposure

5.1.1 Acute Reference Dose (RfD) established for females 13-50 years of age

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: In a developmental toxicity study (MRID 00161316), ziram technical (98% a.i.) in 1% aqueous methyl cellulose was administered by gavage to pregnant New Zealand White rabbits (16/dose) at concentrations of 0, 3, 7.5, or 15 mg/kg/day on GDs 7 through 19. Does were sacrificed on GD 28.

One high-dose doe died on GD 23 and one mid-dose doe died on GD 13. Additionally, one control doe and one mid-dose doe were sacrificed *in extremis* on GDs 14 and 15, respectively; clinical signs observed prior to death in these two animals included weight loss, anorexia, and wheezing. These deaths were not considered to be the result of treatment due to the lack of a dose-response relationship. No other premature deaths occurred and no treatment-related clinical signs of toxicity were observed at any dose level.

At 15 mg/kg, decreased body weights were observed over GDs 0-28 (↓3-17%, $p \leq 0.01$ on GD 10 only). Additionally, for the overall treatment interval (GDs 7-19) and overall study interval (GDs 0-28) body weight gain, as calculated by the reviewers, were reduced as compared to the control (treatment, ↓81%; study, ↓30%, not analyzed for statistical significance). Decreases ($p \leq 0.01$) in absolute (g/animal/day) food consumption were observed beginning at the GDs 7-10 interval (↓19%) and continuing throughout the GDs 13-16 interval (↓44-49%); decreased consumption was also observed for the GDs 16-19 interval (↓24%, not statistically significant [NS]). Food consumption was reduced for the overall treatment interval (↓34%, GDs 7-19) and for the overall study interval (↓18%, GDs 0-28).

At 7.5 mg/kg, decreased body weight gain was observed over GDs 7-19 (↓30%) and GDs 0-28 (↓19%). No other treatment-related maternal effects were noted at the mid-dose level.

No treatment-related findings were observed at gross necropsy of maternal animals.

The number of implantations/doe and percent male were similar between control and treated groups.

The maternal LOAEL is 7.5 mg/kg/day, based on decreased body weight gain. The maternal NOAEL is 3 mg/kg/day.

Reduced atrium/atria, a minor defect (variation), was observed at the mid- (fetal 2.9%; litter, 14.3%) and high-dose (fetal, 2.8%; litter, 20.0%) levels vs controls (fetal, 0.8%; litter, 6.7%). The HIARC did not considered this as an effect since there was no dose response, there were no statistically significant differences in the incidences at any dose, and the data on this parameter, which is a highly subjective observation, showed a wide-spread variation in the size of the atria (enlarged and reduced) among the control and the treatment groups.

At the high-dose level, increases (NS) as compared to the control were observed in the total number of resorptions/doe (↑88%) and the percent postimplantation loss (↑97%). Additionally, reductions (NS) in the number of live fetuses/doe (↓15%) were noted. Upon skeletal examination, absence of the interparietal bone, a major defect (malformation), was observed at the high-dose level only (fetal, 1.9%; litter, 13.3%) vs 0 controls; since this finding was only observed at the high-dose level and without the %fetal and %litter incidence ranges in the historical data, this malformation was considered equivocally treatment-related.

The developmental LOAEL is 15 mg/kg/day, based upon increased resorptions and post-implantation loss. The developmental NOAEL is 7.5 mg/kg/day.

This developmental toxicity study is classified **acceptable/guideline (§83-3[b])** and does satisfy the guideline requirement for a developmental toxicity study in the rabbit; it would be helpful if historical control data (% fetal and % litter incidences) were provided for reduced atrium/atria and absence of the interparietal bone.

Dose and Endpoint for Establishing RfD (Females 13-50): Developmental NOAEL = 7.5 mg/kg/day based on increased resorptions and post-implantation loss at 15 mg/kg/day (LOAEL).

Uncertainty Factor (UF): 100

Comments about Study/Endpoint/Uncertainty Factor: Many fetal effects are presumed to occur from a single dose (acute exposure), and since they occur *in utero*, the selected endpoint is appropriate for this population subgroup (♀ 13-50). The rabbit developmental study was chosen since the LOAELs for both the rabbit and rat developmental studies were the same (16

vs. 15 mg/kg/day, respectively) and the rabbit developmental endpoint was deemed to be more robust and appropriate for risk assessment than the rat developmental endpoint, diaphragmatic thinning. Although diaphragmatic thinning *per se* is a reasonable endpoint for risk assessment, it was not selected in this instance because diaphragmatic hernia, a more severe expression of a related effect, was observed in the control and low mid-dose groups only. As a result, the significance of diaphragmatic thinning at 15 mg/kg/day as an endpoint for risk assessment was questionable.

Acute RfD	=	7.5 mg/kg	= **0.075 mg/kg**
Females 13-50		100	

5.1.2 Acute Reference Dose (RfD) established for the **general population**

Study Selected: Acute Oral Neurotoxicity/ Rat OPPTS 870.6200 (§81-8)

MRID No.: 43362801

Executive Summary: In an acute neurotoxicity study (MRID 43362801), male and female Sprague-Dawley Crl:CD BR® rats received a single gavage dose of 0, 15, 300, or 600 mg/kg of Ziram (tech., 97.8% a.i.) in corn oil (7.5 mL/kg). The 0, 15, and 300 mg/kg groups consisted of 12 animals/sex, the 600 mg/kg group consisted of 16 animals/sex. Functional observational battery tests (FOB) and motor activity were recorded for all animals. FOB and motor activity evaluations were conducted pretreatment, at the time of peak effect (4 hours post-dosing), and on days 7 and 14. At necropsy, brain weights and dimensions were determined for all animals. Five animals/sex were selected for neuropathological evaluation in the control and 600 mg/kg groups.

Four males and three females in the high-dose group died on day 1; three other high-dose females died on days 2, 4, or 5 and one mid-dose female died on day 2. The cause of these deaths is unknown. Gross observations at necropsy revealed white contents of stomach/intestines (probably from corn oil), stomach distention, and in two high-dose females, emaciation. There were no findings consistent with trauma induced by gavage error. The two severely affected mid-dose males (Nos. 15355 and 15387) that survived the 2-week observation period exhibited cyanosis and hypothermia. However, neither cyanosis nor hypothermia were reported in the animals that died on study.

No effects on body weight were apparent in the low-dose group and transient effects were seen in high-dose males. In the mid-dose males, the mean body weights were significantly ($p<0.01$) lower on days 7 (12%) and 14 (16%) for mid-dose males compared with the control group means. The decreased body weights in the mid-dose group on day 14 were attributed to

two males (Nos. 15355 and 15387), with body weights of 159 g and 161 g, respectively, compared with a control group mean of 321 g. Body weights of females were not affected; however, body weight gain was transiently reduced during days 0 - 7 in both males and females at mid and high-dose.

The most significant and biologically relevant findings of treatment were clinical signs of toxicity and effects observed during FOB and motor activity tests. Although both sexes in the mid- and high-dose groups were affected, several of the findings were limited to or occurred most frequently in two mid-dose males (Nos. 15355 and 15387). Clinical signs were generally seen in the first week of the study (but persisted to day 15 in the two mid-dose males) and included dose-related increased incidences of gait alterations, abnormal respiration, abnormal excreta, and distended abdomen. Cyanosis and enophthalmus were limited to two mid-dose males (No. 15355 and 15387) and were seen on day 8 or later on three occasions. Rales observed on one occasion in one low-dose male cannot be clearly attributed to treatment with the test material.

In the FOB evaluations, all six of the functional domains were affected in the mid- and high-dose groups. In general, the responses occurred approximately 4 hours after dosing and were transient in nature (none persisted to day 7). Notable effects on day 0 included altered posture, palpebral closure (eye lid slightly drooping to shut), altered feces consistency, slight lacrimation, slight to severe salivation, red/crusty deposits around nose and mouth, impaired mobility and altered gait, and decreased body temperature. Impaired gait and ataxia were also noted in males at 15 mg/kg on day 0. During the FOB on day 14, several findings were noted in the two most severely affected mid-dose males (altered posture, altered palpebral closure, enophthalmus, impaired mobility, absent startle response and hindlimb extension). It should be pointed out that some findings in these two animals (gasping, mucous membrane change and color, impaired righting reflex) were not even observed on day 7 or day 0, or in animals in the high-dose group. A low incidence of effects on FOB parameters was seen in the low-dose group. These effects (affecting neuromuscular and CNS activity in 1-2 animals) were minimal and cannot be attributed unequivocally to treatment with the test material because of the subjectivity of the endpoints. Significantly ($p<0.05$) decreased motor activity was seen in mid- and high-dose males and females. Total motor activity and ambulatory activity counts were reduced by as much as 82-87% and 76-87%, respectively, compared with controls. However, complete recovery was observed by day 7 in mid-dose males and females and in high-dose females; high-dose males recovered fully by study day 14. Even though the mean counts were not affected in the mid-dose males on day 14, the total motor activity and ambulatory activity counts for males (Nos. 15355 and 15387) were lower than the respective controls and lower than their day 7 values.

There was a dose-related decrease in absolute brain weights which was statistically significant at 300 and 600 mg/kg. No treatment-related effects on brain dimensions were noted. No treatment-related lesions were observed in central or peripheral nervous system tissues examined from the control or high-dose group.

The LOAEL is 15 mg/kg, based on ataxia and slight impairment of gait in males. No NOAEL was determined.

This study is classified as **Acceptable-Guideline** and satisfies the guideline requirements for an acute neurotoxicity study (81-8) in rats.

Dose and Endpoint for Establishing RfD (Gen. Population): 15 mg/kg/day based on ataxia and slight impairment of gait in males.

Uncertainty Factor (UF): 300

Comments about Study/Endpoint/Uncertainty Factor: An additional uncertainty factor of 3 was applied because of the use of a LOAEL (No NOAEL in this study). A 3X was chosen as compared to a 10X since two repeated dose subchronic tests (MRIDs 42450301, 43413701) did not show ataxia in rats at much higher dosages and the most pertinent route of exposure is ingestion of residues on food, not ingestion of the compound alone (the acute study is a gavage study).

Acute RfD (Gen. Pop'n)	$= \frac{15 \text{ mg/kg}}{300}$	=	**0.05 mg/kg**

5.1.3 Chronic Reference Dose (RfD)

Study Selected: Chronic Oral Toxicity/ Dog OPPTS 870.4100 (§83-1b)

MRID No.: 42823901

Executive Summary: In a chronic feeding study (MRID No. 428239-01), Ziram (98.5%; Lot No. 8331 AA) was administered for 52 weeks in the diet to four male and four female beagle dogs per dose at concentrations of 0, 50, 185, and 700 ppm (700 ppm dose reduced to 500 ppm at day 3 of week 12), equivalent to doses of 0, 1.6, 6.6, 17.4 mg/kg/day for males and 1.9, 6.7, and 20.6 mg/kg/day for females, respectively.

There was a treatment-related convulsive episode at week 11 for a female in the 700/500-ppm dose group that required the animal to be euthanitized. In addition to the convulsive episode, the findings for the 700/500-ppm dose group include:1) decreased body weight gain (↓81%) in females over the treatment period and 2) histologic findings for livers (aggregates of Kupffer cells and macrophages, increased foci of degenerate hepatocytes, infiltration of inflammatory cells around central veins and branches of the hepatic vein and portal areas, and increased centrilobular fibrocytes in males). The findings for the 185-ppm dose group include

decreased body weight gain (↓69%) in females during the treatment period. **The NOAEL is 50 ppm based on the lack of significant toxicological effects. The LOAEL is 185 ppm based on decreased body weight gain in females.**

Classification: This study is classified as **acceptable**. The study satisfies most of the guideline requirements for a chronic feeding study in beagle dogs (§83-1).

Dose and Endpoint for Establishing RfD: 1.6 mg/kg/day based on decreased body weight gain in females.

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: This study is of the appropriate route and duration of exposure, and the endpoint, decreased body weight gain, is also observed in other oral and dermal studies.

Chronic RfD = $\frac{1.6 \text{ mg/kg/day}}{100}$ = **0.016 mg/kg/day**

5.2 Occupational/Residential Exposure-Dermal Exposure

Incidental Oral Exposure

There is no expected exposure to small children given the pattern of use with ziram and no likelihood that uses will be expanded that would result in such exposure, so no incidental oral endpoints were selected.

Dermal Absorption

Dermal Absorption Factor: A dermal absorption study is available, but considered unacceptable. Dermal absorption was estimated using the LOAEL of 1000 mg/kg/day in the 21-day dermal toxicity rabbit study (MRID 41297001) and the LOAEL of 7.5 mg/kg/day in the rabbit oral developmental study (MRID 00161316), both studies having the same endpoint - body weight decrement.

$$\frac{7.5}{1000} \times 100 = 0.75\ \% \text{ or} \sim 1\ \% \text{ dermal absorption factor}$$

5.2.1 Short-Term Dermal (1-30 days) Exposure

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: See Acute RfD (Section 2.1).

Dose/Endpoint for Risk Assessment: Developmental NOAEL = 7.5 mg/kg/day based on increased resorptions and post-implantation loss at 15 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This study is of the appropriate duration of exposure. The dermal study was not chosen since it did not evaluate developmental toxicity which is of concern due to the residential uses which may potentially result in exposure of pregnant women to ziram. The rabbit developmental study was chosen since the LOAELs for both the rabbit and rat developmental studies were the same (16 vs. 15 mg/kg/day, respectively) and the rabbit developmental endpoint was deemed to be more robust and appropriate for risk assessment than the rat developmental endpoint, diaphragmatic thinning. Although diaphragmatic thinning *per se* is a reasonable endpoint for risk assessment, it was not selected in this instance because diaphragmatic hernia, a more severe expression of a related effect, was observed in the control and low mid-dose groups only. As a result, the significance of diaphragmatic thinning at 15 mg/kg/day as an endpoint for risk assessment was questionable. A dermal absorption factor of 1% should be applied.

5.2.2 Intermediate-Term Dermal (30 Days to 6 Months) Exposure

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: See Short-term Dermal (Section 2.3.2)

Dose/Endpoint for Risk Assessment: Developmental NOAEL = 7.5 mg/kg/day based on increased resorptions and post-implantation loss at 15 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The rabbit developmental study was chosen for this duration of exposure since it is protective of developmental effects that occur at lower doses than systemic effects in other oral studies of this exposure duration. Also, see comments in Section 2.3.3. A dermal absorption factor of 1% should be applied.

5.2.3 Long-Term Dermal (Several Months to Life-Time)

Study Selected: Chronic Oral Toxicity/ Dog OPPTS 870.4100 (§83-1b)

MRID No.: 42823901

Executive Summary: See Chronic RfD (Section 2.2)

Dose/Endpoint for Risk Assessment: 1.6 mg/kg/day based on decreased body weight gain in females.

Comments about Study/Endpoint: There is no long-term dermal study. The chosen study is of the appropriate duration for this exposure period. See additional comments in the Chronic RfD section (Section 2.2). A dermal absorption factor of 1% should be applied.

5.3 Occupational/Residential Exposure-Inhalation Exposure

5.3.1 Short-Term Inhalation (1-30 days) Exposure

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: See Short-term Dermal (Section 2.3.3)

Dose/Endpoint for Risk Assessment: Developmental NOAEL = 7.5 mg/kg/day based on increased resorptions and post-implantation loss at 15 mg/kg/day (LOAEL).

Comments about Study/Endpoint: No appropriate inhalation study is available. The selected study is of the appropriate duration of exposure. See comments in Section 2.3.3. An inhalation absorption factor of 100% should be applied.

5.3.2 Intermediate-Term Inhalation (30 Days to 6 Months) Exposure

Study Selected: Prenatal Oral Developmental/ Rabbit OPPTS 870.3700 (§83-3b)

MRID No.: 00161916

Executive Summary: See Short-term Dermal (Section 2.3.3)

Dose/Endpoint for Risk Assessment: Developmental NOAEL = 7.5 mg/kg/day based on increased resorptions and post-implantation loss at 15 mg/kg/day (LOAEL).

Comments about Study/Endpoint: No appropriate inhalation study is available. The rabbit developmental study was chosen for this duration of exposure since it is protective of developmental effects that occur at lower doses than systemic effects in other oral studies of this exposure duration. Also, see comments in Section 2.3.3. An inhalation absorption factor of 100% should be applied.

5.3.3 Long-Term Dermal (6 Months to Life-Time) Exposure

Study Selected: Chronic Oral Toxicity/ Dog OPPTS 870.4100 (§83-1b)

MRID No.: 42823901

Executive Summary: See Chronic RfD (Section 2.2)

Dose/Endpoint for Risk Assessment: 1.6 mg/kg/day based on decreased body weight gain in females.

Comments about Study/Endpoint: No appropriate inhalation study is available. The chosen study is of the appropriate duration for this exposure period. See additional comments in the Chronic RfD section (Section 2.2). An inhalation absorption factor of 100% should be applied.

5.4 Margins of Exposure for Occupational/Residential Risk Assessments

A MOE of 100 is required for all durations of occupational dermal and inhalation exposure risk assessments. The MOEs for residential exposure risk assessment will be determined by the FQPA Safety Factor Committee.

5.5 Recommendation for Aggregate Exposure Risk Assessments

For **short- and intermediate-term** exposure risk assessment, the aggregate dermal and inhalation exposure risk assessments are appropriate due to the common toxicological endpoints (developmental - increased resorptions and post-implantation loss) seen in the oral study chosen for these two routes.

For **long-term** exposure risk assessment, the aggregate oral, dermal and inhalation exposure risk assessments are appropriate due to the common toxicological endpoint (decreased body weight gain) seen in the oral study chosen for these three routes.

5.6 Carcinogenic Potential

5.6.1 Conclusions

On February 9, 2000, the Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of ziram. The studies evaluated included a 2-year combined chronic

toxicity/carcinogenicity study in CD (SD) BR rats, a 24-month carcinogenicity study in CD-1 mice as well as NTP studies in F344 rats and $B6C3F_1$ mice. The CARC concluded that Ziram was carcinogenic to CD (SD) BR and F344 male rats and $B6C3F_1$ female mice and was not carcinogenic to CD-1 mice.

5.6.2 Classification of Carcinogenic Potential

In accordance with the Agency's *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified ziram into category "Likely to be carcinogenic to humans" based on the occurrence of C-cell thyroid tumors and hemangiomas in male rats and lung tumors in female mice.

5.6.3 Quantification of Carcinogenic Potential

The Committee recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on C-cell thyroid tumors in male rats. This approach is supported by the findings of benign lung tumors in female mice, lack of mode of action data, and the mutagenicity evidence for ziram.

6.0 FQPA CONSIDERATIONS

6.1 Special Sensitivity to Infants and Children

The FQPA Safety Factor Committee (FQPA, 2001) recommended that the FQPA safety factor for protection of infants and children is necessary when assessing the risk posed by ziram since:

- there is quantitative evidence of increased susceptibility in the developmental neurotoxicity study in rats; and
- there are data gaps in the developmental neurotoxicity study with ziram (morphometric analysis); as well as for a dominant lethal study requested by the CARC.

However the safety factor could be **reduced to 3x** for ziram because:

- There is no quantitative or qualitative evidence of increased susceptibility following *in utero* exposure to rats and rabbits and following pre-/postnatal exposure to rats in the standard developmental and reproduction studies with ziram;
- With respect to the data gaps identified in the toxicity data base for ziram, the outstanding data from the DNT (morphometric analysis) and the results of the dominant lethal study may confirm and characterize the effects seen with ziram but not increase the concern for the effects; and

- The dietary (food and drinking water) and residential exposure assessments will not underestimate the potential exposure for infants, children, and/or women of childbearing age.

The safety factor is required for **All Population Subgroups** when assessing **Dietary and Residential Exposures of All Durations** since there is quantitative evidence of increased susceptibility in the developmental neurotoxicity study in rats.

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8.0 APPENDICES

Tables for Use in Risk Assessment

8.1 Toxicity Profile Summary Tables

8.1.1 Acute Toxicity Table - See Section 4.1

8.1.2 Subchronic, Chronic, and Other Toxicity Table

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity-rat	42450301 (1992) Acceptable/guideline 0, 100, 300, 1000 ppm M: 0, 7.4, 21.4, 67.8 mg/kg/day F: 0, 8.8, 24.2, 76.9 mg/kg/day	NOAEL = [M: 7.4, F: 8.8] mg/kg/day LOAEL = [M: 21.4, F: 24.2] mg/kg/day based on decreases in body weight, body weight gain, food consumption, and minimal histopathological changes in the female liver
870.3150 90-Day oral toxicity-dog	Requirement fulfilled by Chronic dog study-870.4100b	N/A
870.3200 21/28-Day dermal toxicity-rabbit	41297001 (1989) Acceptable/guideline M&F: 0, 100, 300, 1000 mg/kg	NOAEL = [M: >1000, F: 300] mg/kg LOAEL = [F: 1000] mg/kg based on decreased body weight and food consumption and clinical chemistry suggestive of minimal hepatotoxicity. A LOAEL was not observed in males.
870.3250 90-Day dermal toxicity	NA	NA
870.3465 90-Day inhalation toxicity	NA	NA
870.3700a Prenatal developmental-rat	41908701 (1990) Acceptable/guideline F: 0, 1, 4, 16, 64 mg/kg/day	**Maternal** NOAEL = [4] mg/kg/day LOAEL = [16] mg/kg/day based on decreased body weights, reduced food consumption, salivation, and increased water intake. **Developmental** NOAEL = [4] mg/kg/day LOAEL = [16] mg/kg/day based diaphragmatic thinning.

(Table concludes)

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3700b Prenatal developmental-rabbit	00161316 (1986) Acceptable/guideline F: 0, 3, 7.5, 15 mg/kg/day	**Maternal** NOAEL = [3] mg/kg/day LOAEL = [7.5] mg/kg/day based on decreased body weight gain. **Developmental** NOAEL = [3] mg/kg/day LOAEL = [7.5] mg/kg/day based on increased incidence of reduced atrium/atria.
870.3800 Reproduction and fertility effects-rat	43935801 (1996) Acceptable/guideline 0,72, 207, 540 ppm F_0 males: 0, 5.3, 14.8, 37.5 mg/kg/day F_0 females: 0, 6.1, 16.8, 42.8 mg/kg/day F_1 males: 0, 5.6, 16.7, 42.7 mg/kg/day F_1 females: 0, 6.3, 18.4, 47.5 mg/kg/day	**Parental/Systemic** NOAEL = [14.8] mg/kg/day LOAEL = [37.5] mg/kg/day based on reduced body weights, body weights gains, and food consumption in the F_0 and F_1 males and females. **Offspring** NOAEL = [16.8] mg/kg/day LOAEL = [42.8] mg/kg/day based on decreased body weights at birth in F_2 pups and during lactation in F_1 and F_2 pups.
870.4100a Chronic toxicity-CD rat	43404201 (1994) Acceptable/guideline 0, 60, 180, 540 ppm M: 0, 2.5, 7.7, 23.7 mg/kg/day F: 0, 3.4, 10.2, 34.6 mg/kg/day	NOAEL = not established LOAEL = [M: 2.5, F: 3.4] mg/kg/day based on histopathological findings in various organs.
870.4100a Chronic toxicity- F344 rat	NTP (1983) Acceptable/guideline 0, 300, or 600 ppm M: 0, 11, or 22 mg/kg/day F: 0, 13,or 26 mg/kg/day	NOAEL = [M: 22, F: 26] mg/kg/day based on lack of effect. LOAEL = [M: >22, F: >26] mg/kg/day
870.4100b Chronic toxicity- dog	42823901 (1993) Acceptable/guideline 0, 50, 185, 700 ppm M: 0, 1.6, 6.6, 17.4 mg/kg/day F: 0, 1.9, 6.7, 20.6 mg/kg/day	NOAEL = [M: 1.6, F: 1.9] mg/kg/day LOAEL = [M: 17.4, F: 6.7] mg/kg/day based on decreased body weight gain in the females and liver histopathology in males.
870.4200a Carcinogenicity-CD rat	Same as chronic toxicity-rat above (870.4100a).	**Evidence of carcinogenicity** based on increased incidence of benign hemangiomas in CD male rats at 23.7 mg/kg/day
870.4200a Carcinogenicity-F344 rat	Same as chronic toxicity-F344 rat above (870.4100a).	**Evidence of carcinogenicity** based on increased incidence of thyroid C-cell carcinomas and combined adenomas/carcinomas in male rast at 22 mg/kg/day.

(Table concludes)

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.4200b chronic/Carcinogenicity- CD-1 mouse	43373701 (1994) Acceptable/guideline 0, 29, 75, 225, 675 ppm M: 0, 3, 9, 27, 82 mg/kg/day F: 0, 4, 11, 33, 95 mg/kg/day	NOAEL = [M: 9, F: 11] mg/kg/day LOAEL = [M: 27, F: 33] mg/kg/day based on decreased absolute brain weight in both sexes and increased incidence of urinary bladder epithelial hyperplasia and decreased body weight gain in males. **No evidence of carcinogenicity**
870.4200b Chronic/Carcinogenicity- B6C3F1 mouse	NTP (1983) Acceptable/guideline 0, 600, or 1200 ppm M: 0, 122, or 196 mg/kg/day F: 0, 131, 0r 248 mg/kg/day	NOAEL = [M:196]mg/kg/day; not established for females; LOAEL = [M: >196, F: 131] mg/kg/day based on increased incidence of alvelolar epithelial hyperplasia in females **Evidence of carcinogenicity** based on increased incidence of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas in female B6C3F1 mice at ≥131 mg/kg./day
Gene Mutation 870.5265 Salmonella/ mammalian activation gene mutation assay	00147462 (1984) Acceptable/guideline 41642901 (1990) Acceptable/guideline Haworth, et al. (1983) Acceptable/guideline	The test article was positive for gene mutation induction in strain TA100 (±S9). The test article was mutagenic when tested above 50 µg/plate (+S9). The test article was positive in strains TA100 (±S9) and TA1535 (+S9)
Cytogenetics 870.5375 *in vitro* mammalian cytogenetics assay	41287802 (1989) Acceptable/guideline Gulati (1989) Acceptable/guideline	There was no evidence of structural chromosomal aberrations over background. The test article was positive for chromosomal aberrations (±S9).
870.5300 mammalian cell gene mutation assay	McGregor, et al. (1988) Acceptable/guideline	The test article was positive for gene mutation induction (-S9).
870.5395 *in vivo* mammalian cytogenetics assay	The CARC has requested a dominant lethal study.	NA

(Table concludes)

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
Other Genotoxicity 870.5550, Unscheduled DNA synthesis	41287801 (1989) Acceptable/guideline	There was no evidence that unscheduled DNA synthesis was induced.
870.6200a Acute neurotoxicity screening battery	43362801 (1994) Acceptable/guideline M&F: 0, 15, 300, 600 mg/kg	NOAEL = not established LOAEL = [M&F: 15] mg/kg/day based on ataxia and slight impairment of gait in males.
870.6200b Subchronic neurotoxicity screening battery	43413701 (1994) Acceptable/guideline 0, 72, 207, 540 ppm M: 0, 5, 14, 34 mg/kg/day F: 0, 6, 16, 40 mg/kg/day	**Systemic** NOAEL = [M: 14, F: 16] mg/kg/day LOAEL = [M: 34, F: 40] mg/kg/day based on decreased body weight and body weight gains. **Cholinesterase** NOAEL = [M: 14, F: 6] mg/kg/day LOAEL = [M: 34, F: 16] mg/kg/day based on brain cholinesterase inhibition in both sexes and brain neurotoxic esterase activity in the males.
870.6300 Developmental neurotoxicity	43935801 (1996) Unacceptable/guideline 0, 72, 207, 540 ppm Maternal gestation: 0, 5, 13, 32 mg/kg/day Maternal lactation: 0, 11, 30, 79 mg/kg/day	**Maternal** NOAEL = [13] mg/kg/day LOAEL = [32] mg/kg/day based on reduced body weights and/or body weights gains, and decreased food consumption during gestation and lactation. **Offspring** NOAEL = not established LOAEL = [5] mg/kg/day based on increased motor activity.
870.7485 Metabolism and pharmacokinetic s-rat	42391001 (1992) Unacceptable/guideline M&F: 15, 352 mg/kg or 15 mg/kg/day	The test material was rapidly absorbed and excreted via the urine and expired air, and significant amounts were excreted in the feces. Small amounts were widely distributed in the body. Metabolites were not identified.
870.7600 Dermal penetration	Same as 21-day dermal rabbit (870.3200) and rabbit oral developmental (870.7600).	The test material was minimally absorbed.
Special studies	There were no special studies	NA

8.2 Summary of Toxicological Dose and Endpoints for Ziram for Use in Human Risk Assessment

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Females 13 +)	NOAEL = 3 UF = 100	Increased incidence of reduced atrium/atria.	Prenatal Oral Developmental / Rabbit
		Acute RfD (Females 13 +) = 0.03 mg/kg/day	
Acute Dietary (Gen. Population)	LOAEL = 15 UF = 300	Ataxia and slight impairment of gait.	Acute Oral Neurotoxicity / Rat
		Acute RfD (Gen. Population) = 0.05 mg/kg/day	
Chronic Dietary	NOAEL = 1.9 UF = 100	Decreased body weight gain.	Chronic Oral Toxicity / Dog
		Chronic RfD = 0.016 mg/kg/day	
Dermal, Short-Term	NOAEL = 300	Decreased body weight and food consumption. Clinical changes suggestive of minimal hepatotoxicity.	21-Day Dermal Toxicity / Rabbit
Dermal, Intermediate-Term	NOAEL = 300	Decreased body weight and food consumption. Clinical changes suggestive of minimal hepatotoxicity.	21-Day Dermal Toxicity / Rabbit
Dermal, Long-Term[1]	NOAEL = 1.6	Decreased body weight gain.	Chronic Oral Toxicity / Dog
Inhalation, Short-Term[2]	NOAEL = 3	Increased incidence of reduced atrium/atria.	Prenatal Oral Developmental / Rabbit
Inhalation, Intermediate/ Long-Term[2]	NOAEL = 1.6	Decreased body weight gain.	Chronic Oral Toxicity / Dog

[1] Use the appropriate dermal absorption factor (1%) since the NOAEL is from an oral study.
[2] Use the appropriate inhalation absorption factor (100%) since the NOAEL is from an oral study.

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037400

Chemical:	**Zinc, bis(dimethylcarbamodithioato-S,S')**
PC Code:	**034805**
HED File Code	**13000 Tox Reviews**
Memo Date:	**09/25/2001**
File ID:	**TX050160**
Accession Number:	**412-02-0281**

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